412 Rec'd PCT/PTO 23 MAY 2000

Form PTO-1390 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: GC500-2-US

U.S. APPLICATION NO. (If known, see 37 CFR 1.5) [please provide]: 09/554992

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US98/27629	23 December 1998	24 December 1997

TITLE OF THE INVENTION

An Improved Method of Assaying for a Preferred Enzyme and/or Preferred Detergent Composition

APPLICANT(S) FOR DOE/EO/US

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Applicant herewith submits to the United States Designated/Elected Office (do/eo/us) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT submission of items concerning a filing under 35 U.S.C.** 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(l).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☐ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor/s (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16 below concern document/s or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included
13. ☐ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary Amendment
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☐ Other items or information:
    - WO Publication WO 99/34011;
    - International Search Report
    - Written Opinion

U.S. APPLICATION NO. (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
[please provide] 09/554992	PCT/US98/27629	GC500-2-US

17. ☒ The following fees are submitted:		CALCULATIONS PTO USE ONLY
BASIC NATIONAL FEE (37 CFR 1.492(2)(1)-(5)):		
Search Report has been prepared by the EPO or JPO	$ 840	
International preliminary examination fee paid to USPTO (37 CFR 1.482)	$	
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(2)(2))	$	
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(2)(2)) paid to USPTO	$	
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4)	$	
ENTER APPROPRIATE BASIC FEE AMOUNT =		$ 840
Surcharge of $130 for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(4)).		$

CLAIMS	NUMBER FILED		NUMBER EXTRA	RATE	
Total Claims	25	- 20 =	5	x $18.00	$ 90
Independent claims	3	- 3 =		x $78.00	$
MULTIPLE DEPENDENT CLAIM(S) (IF APPLICABLE)				+ $250.00	$
TOTAL OF ABOVE CALCULATIONS =					$ 930
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28)					
SUBTOTAL =					$ 930
Processing fee of **$130** for furnishing the English translation later than ☐20 ☐30 months from the earliest claimed priority date (37 CFR 1.492(f)). +					$
TOTAL NATIONAL FEE =					$ 930
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property					$ 40
TOTAL FEES ENCLOSED =					$ 970
				Amount to be refunded:	$
				charged:	$

a. ☐ A check in the amount of $_____ to cover the above fees is enclosed.

b. ☒ Please charge my Deposit Account No. **07-1048** in the amount of **$970** to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **07-1048**. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

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WO 99/34011

PCT/US98/27629

AN IMPROVED METHOD OF ASSAYING FOR A PREFERRED ENZYME AND/OR PREFERRED DETERGENT COMPOSITION

Background of the Invention

Enzymes are a necessary part of many of the detergent compositions that are currently on the market and the inclusion of enzymes in detergent compositions will undoubtedly increase in the future. One of the most important challenges facing a detergent manufacturer today is the identification of new and improved enzymes and detergent compositions. New enzymes can and commonly do include variants of known enzymes.

Several factors can affect the determination of the "improvement" of a new enzyme over an precursor enzyme, i.e., the enzyme itself, the wash conditions, and the detergent composition that the enzyme is to be mixed with. For example, an enzyme that performs well in one detergent composition may not perform as well in another. Similarly, an enzyme and/or detergent composition may perform well under one set of wash conditions, i.e., Japanese, but not another, i.e., North American. However, identifying a new and improved enzyme or detergent composition can be a time consuming task. For example, in the wake of improved technology that can allow a researcher to produce large numbers of variants in a very short time, it has become critical for the researcher to be able to assay those variants rapidly, efficiently and effectively.

Summary of the Invention

The present invention provides a method of assaying for a preferred enzyme including providing a swatch that includes a piece of material and a stain. The stain is then fixed to the material and a smaller swatch can be removed from the swatch. Alternatively, the smaller swatch can be removed from the larger swatch and then the stain can be fixed. Next, an enzyme is applied to the swatch or smaller swatch and they are incubated together.

The method can further include measuring the degree of removal of the stain from the material. The method can also include agitating the smaller swatch and enzyme during incubation. The material can be, for example, cotton, polyester or mixtures of natural and synthetic fibers. The stain can include blood, milk, ink, grass, gravy, chocolate, egg, cheese, clay, pigment, oil, and combinations thereof. The enzyme can be applied to the swatch or smaller swatch in combination with a detergent ingredient.

The present invention also provides a method of assaying for a preferred detergent composition including providing a swatch that includes a piece of material and a stain. The

stain is then fixed to the material and a smaller swatch can be removed from the swatch. Alternatively, the smaller swatch can be removed from the larger swatch and then the stain can be fixed. Next, a detergent composition is applied to the swatch or smaller swatch and they are incubated together.

The method can further include measuring the degree of removal of the stain from the material. The method can also include agitating the swatch or smaller swatch and detergent composition during incubation. The material can be, for example, cotton, polyester or mixtures of natural and synthetic fibers, cellulose and derivatives of cellulose. The stain can include blood, milk, ink, grass, spinach, wine, tea, gravy, chocolate egg, cheese, clay, pigment, oil, and combinations thereof. The detergent composition can be applied to the swatch or smaller swatch in combination with an enzyme.

Brief Description of the Drawing

Figure 1 shows the correlation between the results of testing six protease variants in a tergotometer test according to the method of the present invention.

Detailed Description of the Invention

One aspect of the present invention is directed to a method of assaying for a preferred enzyme that includes providing a swatch of material - a piece of material and a stain - then fixing the stain to the material, optionally removing a smaller swatch from the swatch, applying the enzyme to the swatch or smaller swatch and incubating them.

A further aspect of the invention is directed to a method of assaying for a preferred detergent composition that includes providing a swatch of material that includes a piece of material and a stain, then fixing the stain to the material, optionally removing a smaller swatch from the swatch, applying the detergent composition to the swatch or smaller swatch and incubating them.

Another aspect of the invention is directed to a method of assaying the release of a stain from a blood/milk/ink (BMI)-stained swatch including measuring the absorbance or fluorescence of, for example, the ink, labeled blood or labeled milk in the supernatant after the swatch has been incubated with an enzyme or detergent composition.

In addition, an aspect of the invention includes a method of agitating the microtiter plate to a sufficient degree to assure complete and efficient incubation of the enzyme with the smaller swatch. The method includes applying a plate sealer to the top of the microtite plate and then clamping another lid on top of the plate sealer.

Any enzyme or combination of enzymes may be used in the present invention. Preferred enzymes include those enzymes capable of hydrolyzing substrates, e.g. stains.

These enzymes are known as hydrolases which include, but are not limited to, proteases (bacterial, fungal, acid, neutral or alkaline), amylases (alpha or beta), lipases, cellulases and mixtures thereof. Particularly preferred enzymes are subtilisins and cellulases. Most preferred are subtilisins such as described in U.S. Patent 4,760,025, EP Patent 130 756 B1 and EP Patent Application WO 91/06637, which are incorporated herein by reference, and cellulases such as Multifect L250™ and Puradax™, commercially available from Genencor International. Other enzymes that can be used in the present invention include oxidases such as laccases, transferases, dehydratases, reductases, hemicellulases and isomerases.

A "swatch" is a piece of material such as a fabric that has a stain applied thereto. The material can be, for example, fabrics made of cotton, polyester or mixtures of natural and synthetic fibers. The swatch can further be paper such as filter paper or nitrocellulose or a piece of a hard material such as ceramic or glass. The stain can be blood, milk, ink, grass, tea, wine, spinach, gravy, chocolate egg, cheese, clay, pigment, oil, or mixtures of these compounds.

A "smaller swatch" is a piece of the swatch that has been cut or otherwise removed from the swatch of material either before or after fixing the stain to the swatch and can, for example, fit into the well of a 24, 48 or 96 well microtiter plate. The "smaller swatch" can also be made by applying a stain to a small piece of material. Preferably, the smaller swatch is a piece of fabric with a stain 5/8" in diameter, more preferably, the smaller swatch is 0.25" in diameter.

When, for example, untreated BMI swatches are washed in detergent without bleach, a large portion of the ink is released even without the help of a protease. Adding a protease leads to a small increase in ink release which can be hard to quantify over the large background. The present invention provides a treatment protocol which allows one to control the degree of fixation of a stain. As a result, it is possible to produce swatches which, for example, release varying amounts of ink when washed in the absence of protease. The use of fixed swatches leads to a dramatic improvement of the signal-to-noise ratio in the wash assays. Furthermore, by varying the degree of fixation one can generate stains which give optimum results under the various cleaning conditions.

Swatches having stains of known "strength" on various types of material are commercially available (EMPA, St. Gallen, Switzerland; wfk - Testgewebe GmbH, Krefeld Germany; or Center for Test Materials, Vlaardingen, The Netherlands) and/or can be made by the practitioner (Morris and Prato, Textile Research Journal 52(4):280-286 (1982)). Preferred swatches are a blood/milk/ink (BMI) stain on a cotton-containing fabric, a spinach

stain on a cotton-containing fabric, or grass on a cotton-containing fabric, and chocolate/milk/soot on a cotton-containing fabric.

A stain can be fixed to a material in a number of ways. For example, the swatch can be incubated with a cross-linking agent to fix the stain. The degree of fixing can be affected by, for example, increasing or decreasing the incubation time, varying the temperature at which the incubation takes place, and/or varying the concentration of the chemical. Suitable cross-linking agents for use in the present invention include hydrogen peroxide, bleaching agents, glutaraldehyde, and carbodiimides.

In a preferred embodiment of the invention, a BMI stain can be fixed to cotton with 0.0003 - 0.3% hydrogen peroxide. Other combinations include grass or spinach fixed with 0.001-1% glutaraldehyde, gelatin and Coomassie stain fixed with 0.001-1% glutaraldehyde, or chocolate, milk and soot fixed with 0.001-1% glutaraldehyde.

An important aspect of the present invention is that the swatch and enzyme and/or detergent formulation must be well agitated during incubation. We have observed that the wash performance data is dependent on the orientation of the swatches in the wells (horizontal versus vertical), particularly in the 96-well plate. This would indicate that mixing was insufficient during the incubation period. Although there are a number of ways to ensure sufficient agitation during incubation, a plate holder in which the microtiter plate is sandwiched between two plates of aluminum can be constructed. This can be as simple as placing, for example, an adhesive plate sealer over the wells then clamping the two aluminum plates to the 96-well plate with any type of appropriate, commercially available clamps. It can then be mounted in a commercial incubator shaker. Setting the shaker to about 400 rpm results in very efficient mixing while leakage or cross-contamination is efficiently prevented by the holder.

Trinitrobenzenesulfonic acid (TNBS) can be used to quantify the concentration of amino groups in the wash liquor. This can serve as a measure of the amount of protein that was removed from the swatch (see Cayot and Tainturier, Anal. Biochem. 249:184-0200 (1997)). However, if a detergent or an enzyme sample leads to the formation of unusually small peptide fragments (for example, from the presence of peptidases in the sample) then one will obtain a larger TNBS signal, i.e., more "noise".

The present invention provides another and better way to measure wash performance of blood/milk/ink that is based on ink release. Proteolysis of protein on the swatches leads to the release of ink particles which can be quantified by measuring the absorbance of the wash liquor. The absorbance can be measured at any wavelength between 350 and 800 nm. In a preferred embodiment, the wavelength is measured at 410 nm. or 620 nm. The wash liquor can also be examined to determine the wash performance

on stains containing grass, spinach, gelatin or Coomassie stain. Preferred wavelengths for these stains include and 670nm for spinach or grass and 620nm for gelatin or Coomassie. For example, an aliquot of the wash liquor (typically 100 - 150ul from a 96-well microplate, for example) is removed and placed in a cuvette or multiwell microplate. This is then placed in a spectrophotometer and the absorbance is read at an appropriate wavelength

The performance of samples of variant proteases (produced, for example, according to the disclosure of U.S. Patent Application Ser. No. 322,678) by the method of the present invention using TNBS and ink release detection can be compared. Several of these samples show inflated wash performance when TNBS detection is used (probably due to peptidase contamination) whereas all samples result in indistinguishable signals when the absorbance of the wash liquor was measured.

The present invention can also be used to determine a preferred enzyme and/or detergent composition for dish washing, for example, using a blood/milk/ink stain on a suitable substrate such as cloth, plastic or ceramic.

In a preferred embodiment of the invention, a BMI stain is fixed to cotton by applying 0.3% hydrogen peroxide to the BMI/cotton swatch for 30 minutes at 25°C or by applying 0.03% hydrogen peroxide to the BMI/cotton swatch for 30 minutes at 60°C. Smaller swatches of approximately 0.25" are cut from the BMI/cotton swatch and placed in the wells of a 96 well microtiter plate. Into each well, a known mixture of a detergent composition and an enzyme such as a variant protein is placed. After placing an adhesive plate sealer onto the top of the microtiter plate, the microtiter plate is clamped to an aluminum plate and agitated for 10-300 minutes. At the end of this time, the supernatants are transferred to wells in a new microtiter plate and the absorbance of the ink at 620nm is measured.

In a further preferred embodiment of the invention, a spinach or grass stain is fixed to cotton by applying 0.01% glutaraldehyde to the spinach/cotton swatch or grass/cotton swatch for 30 minutes at 25°C. Smaller swatches of approximately 0.25" are cut from the swatch and placed in the wells of a 96 well microtiter plate. Into each well, a known mixture of a detergent composition and an enzyme such as a variant protein is placed. After placing an adhesive plate sealer onto the top of the microtiter plate, the microtiter plate is clamped to an aluminum plate and agitated for 10-300 minutes. At the end of this time, the supernatants are transferred to wells in a new microtiter plate and the absorbance of the ink at 670nm is measured.

In another preferred embodiment of the invention, a chocolate/milk/soot stain is fixed to cotton by applying 0.01% glutaraldehyde to the chocolate/milk/soot/cotton swatch

30 minutes at 25°C. Smaller swatches of approximately 0.25" are cut from the swatch and placed in the wells of a 96 well microtiter plate. Into each well, a known mixture of a detergent composition and an enzyme such as a variant protein is placed. After placing an adhesive plate sealer onto the top of the microtiter plate, the microtiter plate is clamped to an aluminum plate and agitated for 10-300 minutes. At the end of this time, the supernatants are transferred to wells in a new microtiter plate and the absorbance of the ink at an appropriate wavelength is measured.

Examples

Example I

A. Description of Tergotometer Protocol

A Tergotometer instrument manufactured by United States Testing Company was used. The machine consists of four or six 1.5 liter beakers and agitator spindles which are inserted into the beakers and rotated in a back-and forth manner at a controlled speed, typically 100 RPM, to mimic the type of agitation that occurs in commercial washing machines. The beakers are immersed in a temperature controlled water bath.

Each beaker was filled with one liter of deionized water to which a controlled amount of calcium and magnesium were added to mimic water hardness conditions found in the geography under study. Water hardness for North American conditions was set to 3 - 6 grains per gallon. The water bath was set to 20°C and the temperature of the water in the beakers was allowed to reach equilibrium at the testing temperature.

1 gram of Tide laundry detergent lacking bleach and enzyme (Procter & Gamble, Cincinnati, Ohio) was added to each beaker and allowed to mix for 1 minute while the spindles were rotating at 100 RPM. The enzyme was added to a final concentration of 0.1 micrograms per milliliter and allowed to mix for 1 minute. Blood-Milk-Ink soiled swatches 3" x 4 ½" obtained from EMPA and modified by exposure to 3.0 % hydrogen peroxide for 30 minutes at 20°C and dried, were used. Six soiled swatches were added to each beaker and allowed to incubate for 20 minutes. After the incubation period the swatches were promptly removed from the beakers and rinsed thoroughly with water. The swatches were then placed flat on a clean lab bench to dry. When the swatches were dry, the reflectance of each swatch was measured at 3 different spots on each swatch, using a reflectance spectrophotometer with a small (typically ¼") diameter aperture, capable of reporting results in the standard LAB scale. For BMI, it is sufficient to report only the L value, which correlates with the darkness of the stain. The L values obtained from the swatches in each pot were averaged to obtain the final reported result.

B. Description of 24-well Assay Protocol:

Blood-Milk-Ink swatches were obtained from EMPA and were exposed to 0.03 % hydrogen peroxide for 30 minutes at 60°C, then dried. Circles of ¼" diameter were cut from the dried swatches and placed one per well in a 24 well microplate. 1 gram per liter Tide laundry detergent without bleach and enzyme was prepared in deionized water, and a concentrated stock of calcium and magnesium was added to result in a final water hardness value of 6 grains per gallon. The detergent was allowed to mix for 15 minutes and was then filtered through a 0.2 micron cellulose acetate filter. Enzyme was added to the filtered detergent from a concentrated stock solution to result in a final concentration 1.25 micrograms per milliliter. The enzyme/detergent solution was then added to the appropriate wells of the microplate. The microplate was then sealed to prevent leakage a placed in a holder on an incubated shaker set to 20°C and 400 RPM and allowed to sha for one hour. The plate was then removed from the incubator/shaker and an aliquot of 2(microliters was removed from each well, and the absorbance at 620 nm wavelength was read for each aliquot and reported.

C. Six protease variants were tested according to A and B above. The results are shown in Table 1. The correlation of the data is plotted in Figure 1. The R^2 value is 0.9652.

Table 1

Tergotometer Microswatch

	L Value	Absorbance 620nm
A	45.62	0.066
B	48.815	0.078
C	51.755	0.086
D	49.06	0.076
E	52.915	0.091
F	53.065	0.096

Claims

1. A method of assaying for a preferred enzyme comprising:
   a) providing a swatch of material comprising a piece of material and a stain;
   b) fixing the stain to the material;
   c) applying an enzyme to the swatch; and
   d) incubating the swatch and enzyme.

2. The method of claim 1, further comprising measuring the degree of removal of the stain from the material.

3. The method of claim 1, wherein the enzyme is selected from the group consisting of a protease, a cellulase, an amylase, a laccase, and a lipase.

4. The method of claim 1, wherein the material is selected from the group consisting of a fabric, plastic, glass or ceramic.

5. The method of claim 1, wherein the stain is selected from the group consisting of blood, milk, ink, grass, spinach, gravy, chocolate, egg, cheese, clay, pigment, oil, and combinations thereof.

6. The method of claim 1, wherein the enzyme is applied to the swatch in combination with a detergent ingredient.

7. The method of claim 1, further comprising agitating the swatch and enzyme during incubation.

8. A method of assaying for a preferred detergent composition comprising:
   a) providing a swatch of material comprising a piece of material and a stain;
   b) fixing the stain to the material;
   c) applying a detergent composition to the swatch; and
   d) incubating the swatch and detergent composition.

9. The method of claim 8, further comprising measuring the degree of removal of the stain from the material.

10. The method of claim 8, wherein the material is selected from the group consisting of a fabric, plastic, glass, or ceramic.

11. The method of claim 8, wherein the stain is selected from the group consisting of blood, milk, ink, grass, spinach, gravy, chocolate, egg, cheese, clay, pigment, oil, and combinations thereof.

12. The method of claim 8, wherein the detergent composition is applied to the swatch in combination with an enzyme.

13. The method of claim 12, wherein the enzyme is selected from the group consisting of a protease, a cellulase, an amylase, a laccase, and a lipase.

14. The method of claim 8, further comprising agitating the swatch and detergent composition during incubation.

15. A method of determining the catalytic efficiency of an enzyme comprising:
    a) providing a swatch of material comprising a piece of material and a stain;
    b) applying the enzyme to the swatch;
    c) incubating the swatch and enzyme;
    d) removing the swatch or supernatant; and
    e) measuring a constituent of the stain.

16. The method of claim 15, wherein the enzyme is selected from the group consisting of a protease, a cellulase, an amylase, a laccase, and a lipase.

17. The method of claim 15, wherein the material is selected from the group consisting of a fabric, plastic or ceramic.

18. The method of claim 15, wherein the stain is selected from the group consisting of blood, milk, ink, grass, gravy, chocolate, egg, cheese, clay, pigment, oil, and combinations thereof.

19. The method of claim 15, wherein the enzyme is applied to the swatch in combination with a detergent ingredient.

20. The method of claim 15, further comprising agitating the swatch and enzyme during incubation.

21. The method of claim 15, wherein the constituent is ink from a BMI stain.

22. The method of claim 15, wherein the constituent is labeled blood from a BMI stain.

23. The method of claim 15, wherein the constituent is in the supernatant.

24. The method of claim 15, wherein the constituent is measured by absorbance of the constituent.

25. The method of claim 15, wherein the constituent is measured by the fluorescence of the constituent.

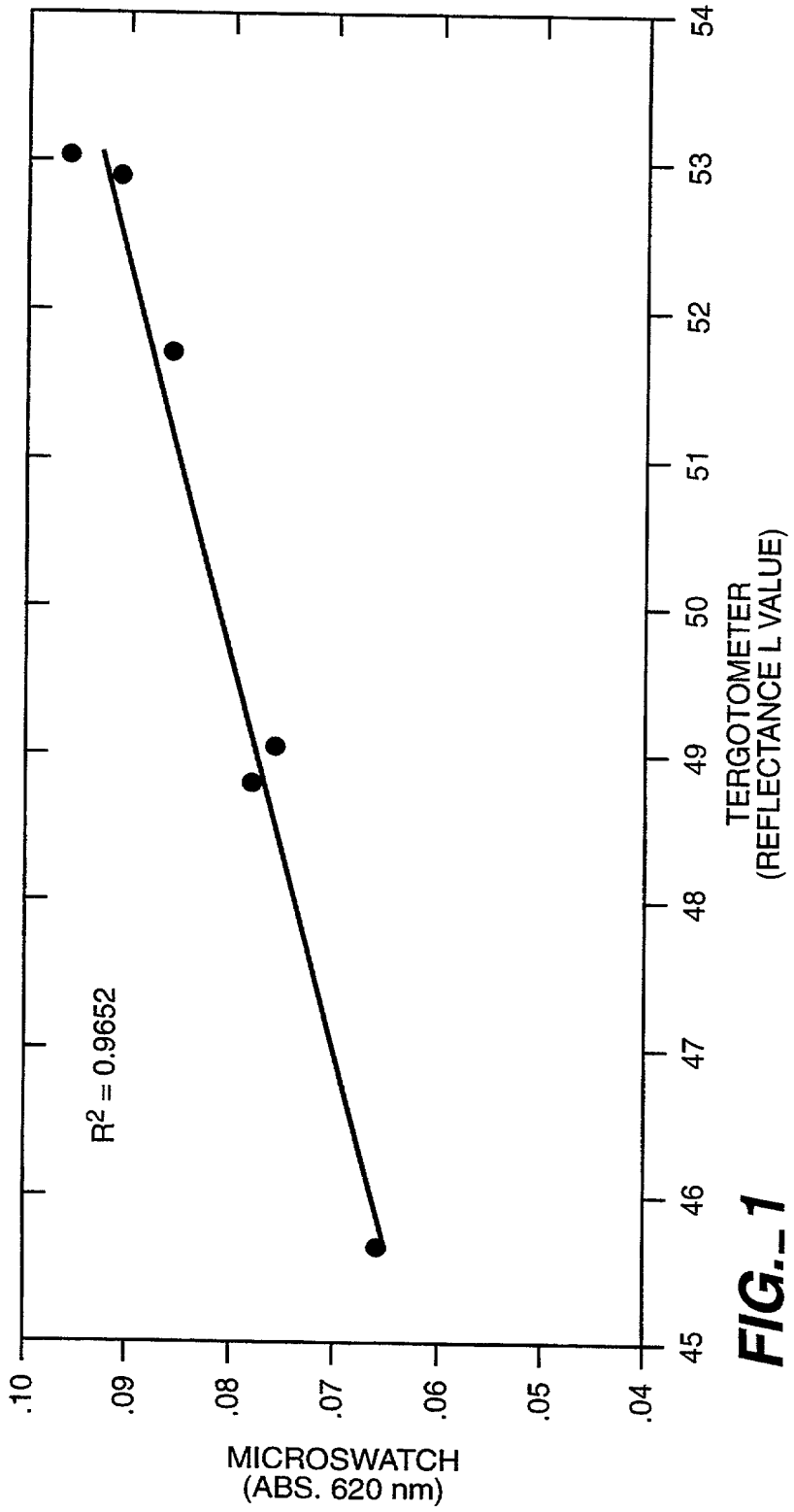
TERGOTOMETER
(REFLECTANCE L VALUE)
54
53
52
51
50
49
48
47
46
45
MICROSWATCH
(ABS. 620 nm)
.10
.09
.08
.07
.06
.05
.04
$R^2 = 0.9652$

FIG._1

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I FURTHER DECLARE THAT ALL STATEMENTS MADE HEREIN OF MY OWN KNOWLEDGE ARE TRUE AND THAT ALL STATEMENTS MADE ON INFORMATION AND BELIEF ARE BELIEVED TO BE TRUE; AND FURTHER THAT THESE STATEMENTS WERE MADE WITH THE KNOWLEDGE THAT WILLFUL FALSE STATEMENTS AND THE LIKE SO MADE ARE PUNISHABLE BY FINE OR IMPRISONMENT, OR BOTH, UNDER SECTION 1001 OF TITLE 18 OF THE UNITED STATES CODE , AND THAT SUCH WILLFUL FALSE STATEMENTS MAY JEOPARDIZE THE VALIDITY OF THE APPLICATION OR ANY PATENT ISSUING THEREON.

SIGNATURE OF INVENTOR **201**	SIGNATURE OF INVENTOR **202**
DATE 5/9/00	DATE 5/10/00
SIGNATURE OF INVENTOR **203**	SIGNATURE OF INVENTOR **204**
DATE 5-9-00	DATE 5/10/00

SIGNATURE OF INVENTOR **205**	
DATE	

I FURTHER DECLARE THAT ALL STATEMENTS MADE HEREIN OF MY OWN KNOWLEDGE ARE TRUE AND THAT ALL STATEMENTS MADE ON INFORMATION AND BELIEF ARE BELIEVED TO BE TRUE; AND FURTHER THAT THESE STATEMENTS WERE MADE WITH THE KNOWLEDGE THAT WILLFUL FALSE STATEMENTS AND THE LIKE SO MADE ARE PUNISHABLE BY FINE OR IMPRISONMENT, OR BOTH, UNDER SECTION 1001 OF TITLE 18 OF THE UNITED STATES CODE , AND THAT SUCH WILLFUL FALSE STATEMENTS MAY JEOPARDIZE THE VALIDITY OF THE APPLICATION OR ANY PATENT ISSUING THEREON.

SIGNATURE OF INVENTOR **201**	SIGNATURE OF INVENTOR **202**
DATE	DATE
SIGNATURE OF INVENTOR **203**	SIGNATURE OF INVENTOR **204**
DATE	DATE

SIGNATURE OF INVENTOR **205** Joanne Nadley	
DATE 5/4/00.	

09654098.060300

DECLARATION
AND POWER OF ATTORNEY

AS A BELOW NAMED INVENTOR, I HEREBY DECLARE THAT:

MY RESIDENCE, POST OFFICE ADDRESS AND CITIZENSHIP ARE AS STATED BELOW NEXT TO MY NAME. I BELIEVE I AM THE ORIGINAL, FIRST AND SOLE INVENTOR (IF ONLY ONE NAME IS LISTED BELOW) OR AN ORIGINAL, FIRST AND JOINT INVENTOR (IF PLURAL NAMES ARE LISTED BELOW) OF THE SUBJECT MATTER WHICH IS CLAIMED AND FOR WHICH A PATENT IS SOUGHT ON THE INVENTION ENTITLED **AN IMPROVED METHOD OF ASSAYING FOR A PREFERRED ENZYME AND/OR PREFERRED DETERGENT COMPOSITION** THE SPECIFICATION OF WHICH

CHECK ONE:

IS ATTACHED HERETO

WAS FILED ON _____ AS APPLICATION SERIAL NO. _____ AND WAS AMENDED ON _____.

I HEREBY STATE THAT I HAVE REVIEWED AND UNDERSTAND THE CONTENTS OF THE ABOVE IDENTIFIED SPECIFICATION, INCLUDING THE CLAIMS, AS AMENDED BY ANY AMENDMENT REFERRED TO ABOVE. I ACKNOWLEDGE THE DUTY TO DISCLOSE INFORMATION WHICH IS MATERIAL TO PATENTABILITY AS DEFINED IN TITLE 37, CODE OF FEDERAL REGULATIONS §1.56.

I HEREBY CLAIM FOREIGN PRIORITY BENEFITS UNDER TITLE 35, UNITED STATES CODE §119, OF ANY FOREIGN APPLICATION(S) FOR PATENT OR INVENTOR'S CERTIFICATE LISTED BELOW AND HAVE ALSO IDENTIFIED BELOW ANY FOREIGN APPLICATION FOR PATENT OR INVENTOR'S CERTIFICATE HAVING A FILING DATE BEFORE THAT OF THE APPLICATION ON WHICH PRIORITY IS CLAIMED.

APPLICATION NUMBER	COUNTRY	DATE OF FILING	PRIORITY CLAIMED YES	NO

I HEREBY CLAIM THE BENEFIT UNDER TITLE 35, UNITED STATES CODE §120, OF ANY UNITED STATES APPLICATION(S) OR PCT INTERNATIONAL APPLICATION(S) DESIGNATING THE UNITED STATES OF AMERICA THAT IS LISTED BELOW AND, INSOFAR AS THE SUBJECT MATTER OF EACH OF THE CLAIMS OF THIS APPLICATION IS NOT DISCLOSED IN THE PRIOR UNITED STATES APPLICATION IN THE MANNER PROVIDED BY THE FIRST PARAGRAPH OF TITLE 35, UNITED STATES CODE §112, I ACKNOWLEDGE THE DUTY TO DISCLOSE MATERIAL INFORMATION AS DEFINED IN TITLE 37, CODE OF FEDERAL REGULATIONS §1.56(A) WHICH OCCURRED BETWEEN THE FILING DATE OF THE PRIOR APPLICATION AND THE NATIONAL OR PCT INTERNATIONAL FILING DATE OF THIS APPLICATION.

APPLICATION NUMBER	DATE OF FILING	STATUS - PATENTED, PENDING OR ABANDONED
60/068,796	24 DECEMBER 1997	ABANDONED
PCT/US98/27629	23 DECEMBER 1998	PENDING

POWER OF ATTORNEY: AS A NAMED INVENTOR I HEREBY APPOINT AS MY ATTORNEY(S) WITH FULL POWER OF SUBSTITUTION AND REVOCATION, TO PROSECUTE THIS APPLICATION AND TRANSACT ALL BUSINESS IN THE PATENT AND TRADEMARK OFFICE CONNECTED THEREWITH:

MARGARET A. HORN, REG. NO. 33,401;
CHRISTOPHER L. STONE, REG. NO. 35,696
JEFFERY D. FRAZIER, REG. NO. 34,601
SUSAN FARIS, REG. NO. 41,739

SEND CORRESPONDENCE TO:	DIRECT TELEPHONE CALLS TO:
SUSAN FARIS GENENCOR INTERNATIONAL, INC. 925 PAGE MILL ROAD PALO ALTO, CALIFORNIA 94304	650 846-7609